Atomic Force Microscopy Indentation of Supported Lipid Bilayers

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Abstract

•In this work, AFM indentation experiments of supported lipid bilayers (SLB) on mica are performed to investigate their hardness. In such measurements, the strain response of the SLB to the compression stress applied by the sharp tip of the AFM probe (curvature radius of 10 nm) is analyzed. It was observed that under the compression stress, the SLB deformed first elastically and then plastically (with a lateral disruption of the lipid bilayer). Transition between elastic and plastic deformation is characterized by the SLB breakthrough force. It is shown that plasma hydroxylation of the AFM tips decreases slightly the SLB breakthrough force.

Material and methods

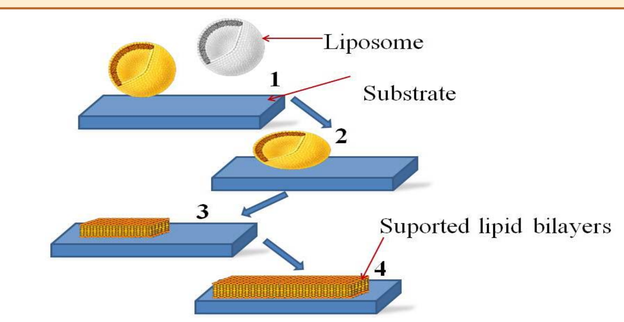

Fig. 1 Schematic representation of preparation of SLB from solution of unilamellar lipid vesicles

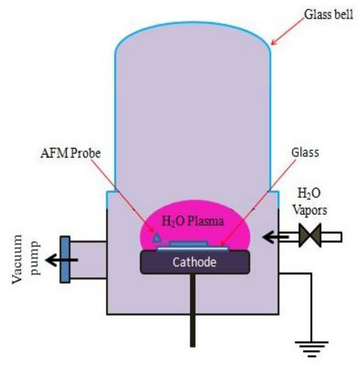

Fig. 2 The plasma reactor used to hydroxylation the AFM probe.

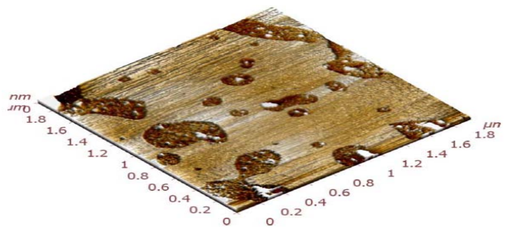

Fig. 3 3D representation of AFM topography AFM image of the SLB on mica

Preparation of Supported Lipid Bilayer (SLBS) samples

❖High purity (>99%) 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipids were purchased from Avanti Polar Lipids (Alabaster, AL, USA) in powder form. Chloroform (>99%) was purchased from Sigma-Aldrich and ethanol absolute (>99.9%) was purchased from EMSURE®. The lipid was dissolved in chloroform to obtain a lipid solution with the concentration of 25mg/mL. The solution was placed in vacuum to evaporate the solvent and obtain a thin lipid film. Then, the thin lipid film was dissolved in a solution of 0,1M NaCl and 10mM HEPES at pH 7 and stirred with a vortex stirrer to form multilamellar vesicles. Finally, the solution was sonicated for few minutes in a heated bath to obtain a solution of unilamellar vesicles. A drop with the volume of 50 μl was deposited on a freshly cleaved mica sheet and left to dry in open air. The liposome in solution are adsorbed on hydrophilic surface of mica to form SLBS (**Fig. 1**),

Hydroxylation of Atomic Force Microscopy probes

❖The AFM indentation experiments were performed with as-received and plasma-hydroxylated AFM tips. Before, plasma hydroxylation, the as-received AFM probes were washed in chloroform (Sigma-Aldrich, ≥98.5%) to remove large dust particles from the surface. Then, the AFM probes were exposed for 3 minutes to the negative glow plasma of a d. c. discharge in a mixture of air and water vapour at 40 Pa. The discharge current intensity and voltage were maintained constant at 3 mA and 470 V, respectively. This treatment removes the surface contaminant molecules from the AFM probe surface and generates surface silanol (Si-OH). After plasma treatment, the AFM probes were used immediately in SLB indentation experiments.

Atomic Force Microscopy measurements

❖The AFM measurements were performed at room temperature in PBS at pH 7 using a commercial AFM apparatus (XE-100 from Park Systems, South Korea). The AFM images were obtained in contact mode in PBS using a commercial AFM probe (MLCT from Bruker) with very flexible silicon nitride cantilevers (nominal force constant of 10 pN/m) and sharpened tips (nominal radius < 10 nm).The force spectroscopy measurements were performed with the same AFM probe using the dedicated controlling software of the microscope. The AFM loading and unloading force-displacement curves were acquired at a constant speed of scanner extension (1□m/s). The cantilever deflection versus scanner extension curves acquired in the experiments were calibrated and transformed in force versus tip displacement (tip-sample distance).

RESULTS AND DISCUSSION

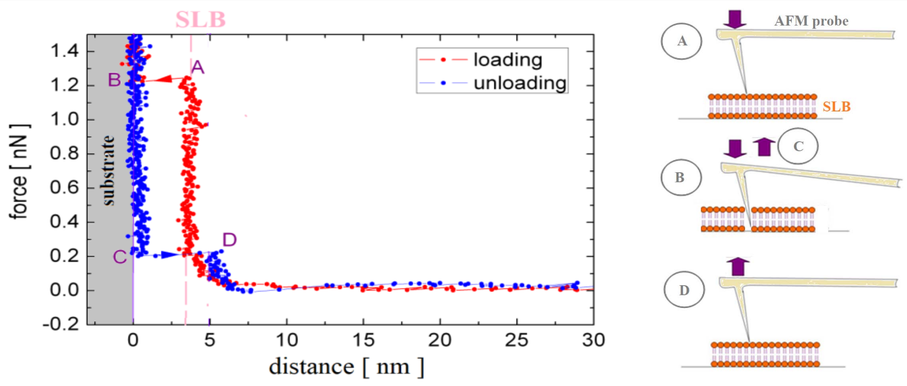

Fig. 4 a) Example of force-distance curve recorded during loading (increasing force) and unloading (decreasing force) applied on the AFM tip in contact with SLB on mica substrate. **b)** Sketch of SLB indentation process: A: The AFM tip is pushed against SLB; B: the AFM tip penetrate the SLB; C: the AFM tip is retracted; D: the SLB reform on substrate.

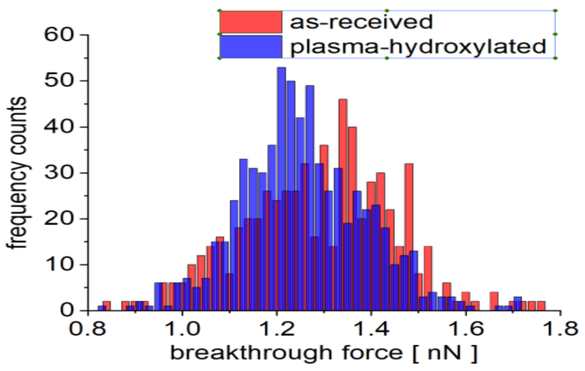

Fig. 5 Histograms of breakthrough force values recorded in force spectroscopy measurements performed with as-received, plasma hydroxylated AFM tips on SLB.

➢Figure 4 shows a typical force-distance curve obtained in SLB indentation experiments. The position of mica surface has been taken as reference in this plot. After the AFM tip is brought in mechanical contact with SLB, a constantly increasing force is applied until the AFM tip suddenly breaks through the lipid bilayers (part A-B on the loading force-distance curve) at a threshold value of the impinging force, an event that separates the elastic from the plastic regimes of SLB deformation. The breakthrough events are easily identified on approach force-distance curves as sudden jumps in the position of the AFM tip (about 4 nm) at constant compression. After the breakthrough, the AFM tip gets in contact with the mica substrate and cannot move further due to the large hardness of the substrate. During the SLB breakthrough, the lipid bilayers collapse under the compression stress applied by the AFM tip, the constant compression force during the event being named breakthrough force. During unloading process, the AFM probe is retracted and the SLB reform quickly under a small compression force (portion C-D on unloading force-distance curve in Fig. 4)

➢AFM force spectroscopy measurements were performed on SLBs with as-received and plasma-hydroxylated AFM tips. Histograms of breakthrough force values obtained in these measurements are presented in Fig. 5. The histograms show slightly larger breakthrough force values for as-received AFM probe (1.4 nN) in comparison with plasma hydroxylated AFM probe (1.2 nN).

CONCLUSION

- ✓ Mechanical properties of the supported lipid bilayers (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine -POPC) was studied by AFM indentation in phosphate buffered saline (PBS) solution.
- ✓ The lipid bilayers are punched by the AFM tip at certain value of compression force applied on the AFM tip, value called breakthrough force.
- ✓ The breakthrough force values depends on AFM tip sharpness, lipid composition and AFM tip surface chemistry
- ✓ In this work we show that hydroxylation of the AFM tips by water vapor plasma lower the SLB breakthrough force